0022-202X/80/7503-0284$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 75:284–287, 1980

Vol. 75, No. 3
Printed in U.S.A.

Suppression of Allergic Contact Dermatitis by α-L-fucose

SHOJI HASEGAWA, M.D., TORU BABA, M.D., AND YOSHIAKI HORI, M.D.

Department of Dermatology, Tokyo University Branch Hospital, Tokyo, Japan (SH and YH) and Department of Dermatology, Institute of Clinical Medicine, University of Tsukuba, Ibaragi, Japan (TB)

We observed that L-fucose could suppress the skin reaction of allergic contact dermatitis induced by dinitrochlorobenzene. This observation was ascertained by histological examination as well as by visual inspection. The degrees of the acanthosis and the spongiosis were significantly slighter in the skin lesions of guinea pigs that received L-fucose injection, than in those of control animals. The number of infiltrated mononuclear cells in epidermis and dermis in the skin lesions was much less in the animals received L-fucose injection than that in the skin lesions of control animals.

On the other hand, L-fucose had no suppressive effect on irritant contact dermatitis induced by high concentration (4%) of dinitrochlorobenzene.

Allergic contact dermatitis is generally accepted as manifestation of cell-mediated immunity. Its histological findings consist of epidermal cell damage and mononuclear cell infiltration in epidermis and dermis. Infiltration of mononuclear cells begins first in the dermis at 3 hr after the induction as shown by light microscopy [1]. On electron microscopic examination, apposition of mononuclear cells, resembling lymphocytes, to the epidermal Langerhans cells is exhibited also at 3 hr [2] and early changes of epidermal cells are revealed at 6 hr [3]. The epidermal cell damage as well as infiltration of mononuclear cells in the epidermis and the dermis become evident at 12 to 24 hr [4] However, the inflammatory mediators leading to these morphological changes still remains unclarified.

L-fucose has been shown capable of inhibiting lymphokine activities *in vitro* system [5–7]. In a previous study [8], we demonstrated that L-fucose can inhibit lymphokine activities *in vivo*; L-fucose inhibited the ability of lymphokine-containing supernatants of lymphocytes culture to induce skin reactions or cause reduction in the macrophage content of peritoneal exudates. L-fucose could inhibit the cutaneous delayed type hypersensitivity reactions of the guinea pigs.

Modification of allergic contact dermatitis by L-fucose, if possible, seems to provide a good clue for clarification of the mediators in the inflammatory processes of allergic contact dermatitis. Then, in the present study the effect of L-fucose on allergic contact dermatitis was studied.

MATERIALS AND METHODS

Induction of Contact Sensitivity

Hartley albino female guinea pigs weighing 350 to 400 gm were used. Contact sensitivity to dinitrochlorobenzene (DNCB) was induced by 11 daily paintings on the clipped nape with 0.5 ml of 2.5% DNCB acetone solution.

Manuscript received October 5, 1979; accepted for publication March 27, 1980.

Dr. Hasegawa's present address is the Department of Dermatology, Kitasato University School of Medicine, Kanagawa, Japan.

Reprint requests to: Shoji Hasegawa, M.D., Department of Dermatology, Kitasato University School of Medicine, 1-15, Kitasato 1 chome, Sagamihara-shi, Kanagawa-ken, 228 Japan.

Abbreviations:
DNCB: dinitrochlorobenzene
MIF: migration inhibitory factor
PBS: phosphate buffered saline

Challenge Testing of Sensitized Animals

Fifteen days later the last painting with DNCB, the guinea pigs were challenged by applying 50 μl of 0.08% DNCB acetone solution on the clipped back. Twenty-four hours after challenging, the degree of skin reaction was described according to scoring system established by Chase [9]. The grading of the inflammatory reactions were: 0, negative; ±, many faint pink small spots or few faint pink spots; 1^+, faint pink; 2^+, pale pink; 3^+, pink but either somewhat pale or macular; 4^+, pink usually very slightly elevated.

Monosaccharides

α-L-fucose and L-arabinose were purchased from Sigma Chemicals, California, USA. These sugars were dissolved in phosphate buffered saline (PBS) at a concentration of 0.5 M.

RESULTS

Effect of L-Fucose on the Skin Reactions of Allergic Contact Dermatitis

A total of 24 guinea pigs sensitized previously with DNCB were divided into 3 groups of 8 guinea pigs each. In our previous study of suppression of lymphokine activities by L-fucose, intravenous (i.v.) injection of 0.5 M L-fucose simultaneously at the time of injection of lymphokines or challenging antigens into the animals provided us successful results [8]. Then, in the present study, effect of L-fucose on allergic contact dermatitis was studied in an identical manner. L-arabinose showed no suppressive effect on lymphokine activities in the previous study and was used as a control sugar in the present study. All of the 3 groups were challenged with 0.08% DNCB acetone solution. The first group was injected intravenously with 0.5 M L-fucose in 1 ml of PBS. The second group was injected intravenously with 0.5 M L-arabinose in 1 ml of PBS simultaneously. No toxicity was noted with L-fucose or L-arabinose preparation used as described previously [8]. The last group received no intravenous injection. The latter two groups served as the controls. The results are shown in Table I. Animals receiving L-fucose injection showed significant reduction of skin reactions with respect to elevation and intensity of erythema. In contrast, the skin reactions of animals that received L-arabinose were not significantly different in intensity from those of noninjected animals as indicated by the scoring system.

Considering the possibility that the degree of sensitization or the intensity of reaction might be unequal among individuals, we explored the ability of L-fucose to suppress allergic contact dermatitis in the same sensitized guinea pigs. Six guinea pigs previously sensitized with DNCB were prepared. First, all the guinea pigs were challenged with DNCB. Forty-eight hours later, they were divided into 2 groups of 3 guinea pigs each. One group of the animals was rechallenged on a different skin site and simultaneously given an injection of L-fucose. As a control, the other group was rechallenged without injection of L-fucose. Suppressive effect of L-fucose on lymphokine activities did not remain in the animals at 48 hr after L-fucose injection (Baba T., Yoshida T., and Cohen S., unpublished data). Then further, 48 hr later, all the 6 animals were challenged on the other different skin site. We were able to achieve a significant reduction in the intensity of skin reaction with intravenous injection, as compared to the first and third challenge. The skin reactions of the second challenge on the control animals showed no significant difference in the intensity as compared to the first and third challenge (Fig 1).

TABLE I. *Effect of L-fucose on the skin reactions in allergic contact dermatitis*[a]

Guinea pig No.[b]	Chase's scores[c]		
	Sugars injected i.v.[d]		
	L-fucose	L-arabinose	Nontreated
1	±[c]	3^+	3^+
2	1^+	3^+	4^+
3	1^+	4^+	4^+
4	1^+	2^+	3^+
5	1^+	3^+	2^+
6	2^+	4^+	4^+
7	±	2^+	2^+
8	2^+	3^+	3^+

[a] Contact sensitivity to DNCB was induced by 11 daily paintings on the clipped nape with 0.5 ml of 2.5% DNCB acetone solution. Fifteen days later the last painting with DNCB, the guinea pigs were challenged by applying 50 μl of 0.08% DNCB acetone solution on the clipped back.

[b] A total of 24 guinea pigs sensitized previously with DNCB were divided into 3 groups of 8 guinea pigs each.

[c] Twenty-four hours after challenging, the degree of skin reaction was described according to scoring system established by Chase.

[d] Monosaccharide solutions (0.5 M) were injected intravenously at the time of challenge tests.

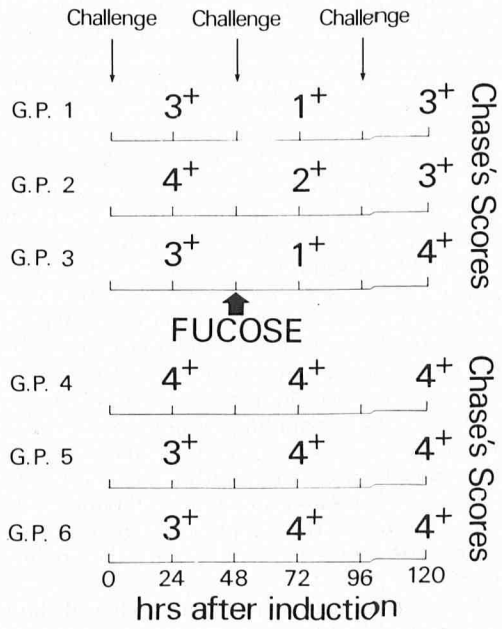

FIG 1. The ability of L-fucose to suppress allergic contact dermatitis in the same sensitized guinea pigs. First, the guinea pigs were challenged by DNCB. Forty-eight hours later they were rechallenged on a different skin site and simultaneously injected intravenously with L-fucose. Further, 2 days later, they were challenged on the other different skin site without intravenous injection. A significant reduction was shown in the intensity of skin reaction with intravenous injection, as compared to the first challenge and the third one (*G.P. 1–3*). *G.P.* 4–6 are noninjected control animals.

Histological Examination of Skin Reactions

Reaction site from individual animals were biopsied and histological examination utilizing sections routinely stained with hematoxylin and eosin was performed. The skin lesions from nontreated guinea pigs showed acanthosis, spongiosis and infiltration of mononuclear cells to epidermis and in dermis. The skin lesions from L-arabinose injected animals were almost the same as those of nontreated animals on histological study. However, compared with these control animals, in the skin lesions of guinea pigs that received L-fucose injection the degrees of the acanthosis and the spongiosis were significantly slighter. Moreover, the number of infiltrated mononuclear cells in epidermis and dermis in the skin lesions was much less in the animals received L-fucose injection than that in the skin lesions of the control animals (Fig 2).

Effect of L-Fucose on Previously Induced Allergic Contact Dermatitis

To study whether simultaneous injection of L-fucose at the time of challenging is necessary for suppression of allergic contact dermatitis, the effect of L-fucose on 6-hr-old allergic contact dermatitis was studied. The early changes of epidermal cells in the allergic contact dermatitis are visible on the electron microscopic study at 6 hr after the induction [3]. Nine guinea pigs previously sensitized with DNCB were prepared and divided into 3 groups. All of the 3 groups were challenged with 0.08% DNCB acetone solution. First group was received simul-

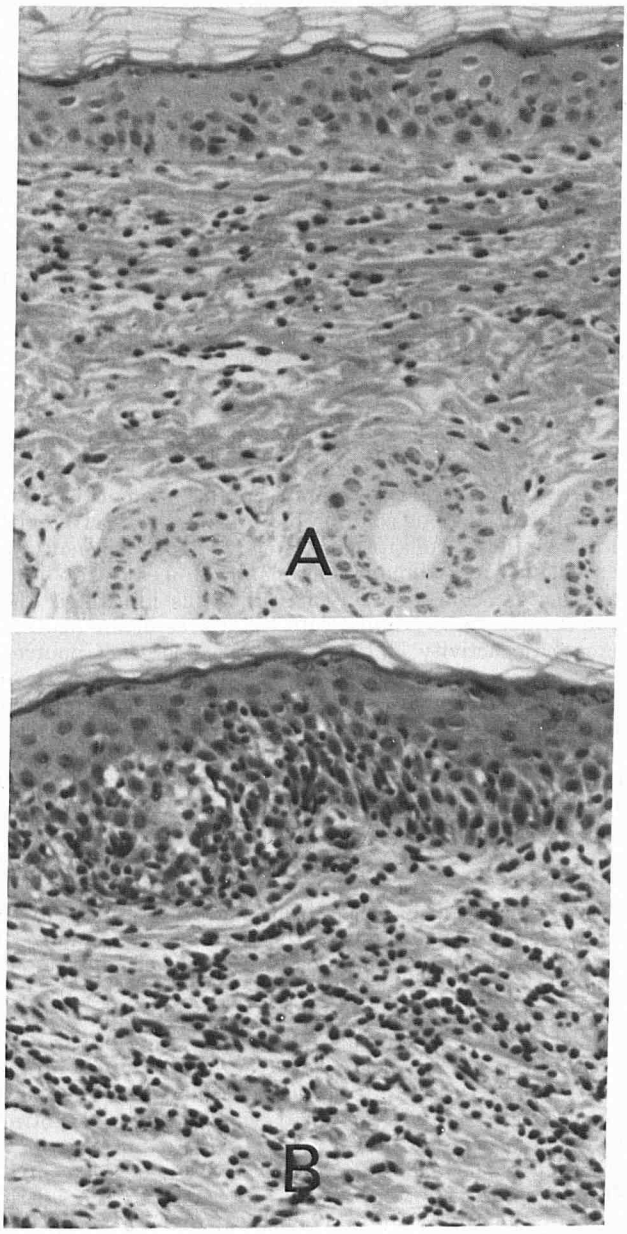

FIG 2. Histological examination of skin reactions (× 100). The degrees of the acanthosis and the spongiosis were significantly slighter in the skin lesions of guinea pigs that received L-fucose injection (*A*), than in those of nontreated animals (*B*). The number of infiltrated mononuclear cells in epidermis and dermis in the skin lesions was much less in the animals received L-fucose injection (*A*) than that in the skin lesions of nontreated animals (*B*).

TABLE II. *Effect of L-fucose on previously induced allergic contact dermatitis*[a]

Guinea pig No.[b]	Chase's scores[c]		
	L-fucose (0.5 M) i.v. injection		
	Simultaneous injection[d]	Six hr after challenging[e]	Nontreated[f]
1	±[c]	1+	3+
2	1+	1+	3+
3	±	±	2+

[a] Contact sensitivity to DNCB was induced by 11 daily paintings on the clipped nape with 0.5 ml of 2.5% DNCB acetone solution. Fifteen days later the last painting with DNCB, the guinea pigs were challenged by applying 50 μl of 0.08% DNCB acetone solution on the clipped back.

[b] A total of 9 guinea pigs sensitized previously with DNCB were divided into 3 groups of 3 guinea pigs each.

[c] Twenty-four hours after challenging, the degree of skin reaction was described according to scoring system established by Chase.

[d] First group was received i.v. injection of L-fucose at the time of challenge tests.

[e] The second group was received i.v. injection of same dose of L-fucose at 6 hr after challenging.

[f] The third group received no i.v. injection.

taneous i.v. injection of 1 ml of 0.5 M L-fucose. The second group was received i.v. injection of same dose of L-fucose at 6 hr after challenging. The third group received no i.v. injection. As shown in Table II, animals receiving L-fucose injection at 6 hr after the challenging showed significant reduction of skin reactions. No significant difference was observed between the skin reactions of the first group which received simultaneous L-fucose injection and those of the second group which received L-fucose injection at 6 hr after the challenging.

Effect of L-Fucose on Irritant Contact Dermatitis Induced by DNCB

In our previous study of irritant contact dermatitis induced by 4% DNCB acetone solution [10], it was demonstrated that irritant contact dermatitis generated C3-dependent neutrophil chemotactic activity in its inflammatory processes at 6 hr after the induction. The number of neutrophils infiltrated in the lesion seemed to be parallel with the degree of the neutrophil chemotactic activity in the lesion; the degree of neutrophil chemotactic activity reached a peak at 12 to 24 hr after the induction, when the lesion was found to be infiltrated predominantly with neutrophils.

Effect of L-fucose on irritant contact dermatitis was studied. Eight guinea pigs were prepared and divided into 2 groups. One group of the animals was received 1 ml of 0.5 M L-fucose i.v. injection simultaneously at the time of induction of irritant contact dermatitis by 4% DNCB acetone solution. Another group received no i.v. injection as a control. The skin reactions showed no significant difference between 2 groups in their intensity of erythema and in their histological findings; the degree of epidermal damage and the number of infiltrated cells in dermis.

Although the attempts were made to suppress the 4% DNCB-induced irritant contact dermatitis by L-fucose with higher concentrations (1 ml of 1 M and 1 ml of 2 M L-fucose respectively), no suppressive effects were obtained. No toxic effect of L-fucose on the animals was noted even with high concentration of L-fucose, such as 1 M or 2 M.

Croton oil, instead of DNCB, was used to induce irritant contact dermatitis. L-fucose did not suppress these irritant contact dermatitis when 1 ml of 0.5 M, 1 M and 2 M respectively was injected.

DISCUSSION

L-fucose, which is monosaccharide, has been shown to be an inhibitor of lymphokine activities from the following observations. Remold has reported that L-fucose blocks the action of macrophage migration inhibitory factor (MIF) [5], and Rocklin has also demonstrated that human lymphocyte MIF activity on human blood monocytes is reduced by L-fucose [6]. Amsden et al have found that L-fucose is able to inhibit the lymphokine activities of macrophage migration inhibition, macrophage chemotaxis and neutrophil chemotaxis, whereas nonlymphokine chemotactic factors such as those derived from complement components or bacterial products are not influenced by L-fucose [7]. In our previous study [8], L-fucose could inhibit, in an *in vivo* assay system, the ability of lymphokine-containing supernatants of lymphocyte culture to induce skin reactions that mimicked a delayed type hypersensitivity reaction or to cause reduction in the macrophage content of peritoneal exudates. Although the lymphokine-free control supernatants induced nonspecific inflammatory reactions, these were not capable of L-fucose inhibition. The Arthus reaction, which has been accepted by Ward and Hill as a manifestation of C5-mediated reaction [11], could not be suppressed by L-fucose. L-fucose had no suppressive effect on histamine-induced vascular permeability (Baba T., Yoshida T. and Cohen S., unpublished data).

In the present study, it was demonstrated that allergic contact dermatitis was suppressed by L-fucose, when previously sensitized animals were challenged with DNCB and simultaneously given an injection of L-fucose. Based on the observations described above, the suppression mechanism of L-fucose on allergic contact dermatitis may be due to inhibitory action of L-fucose on the activity of lymphokines generated in the lesion. Then, we presumed that production of lymphokines occur in allergic contact dermatitis and the lymphokines play a predominant role in the inflammatory processes.

Epidermal Langerhans cells have been demonstrated to have antigen-presenting ability to T-lymphocytes [12] and their macrophage like properties have been also exhibited; Langerhans cells bear Fc and C3 receptors [13] as well as Ia antigen at the surface [14]. In addition, it has been demonstrated that Langerhans cells can induce an antigen-specific T-cell proliferation response comparable in magnitude to that induced by macrophages [12]. On the other hand, Silberberg, Baer, and Rosenthal have exibited on the electron microscopic study the apposition of mononuclear cells, resembling lymphocytes, to Langerhans cells in the epidermis of allergic contact dermatitis as early as 3 hr after the induction [2]. Then, although it has been generally accepted that lymphokine-production of T-lymphocytes is mediated by macrophages, the lymphokines generated in allergic contact dermatitis might be mediated by the epidermal Langerhans cells.

In allergic contact dermatitis, epidermal cell damages are visible at 6 hr on the electron microscopic examination, when the apposition of mononuclear cells to Langerhans cells is also observed [3]. In the present study, L-fucose showed remarkable suppressive effect on the allergic contact dermatitis of animals, when injected at 6 hr. Thus, it seems to be likely that the predominant inflammatory mediators in allergic contact dermatitis after starting the epidermal cell damages are still lymphokines and that modification of inflammatory processes by the epidermal cell damages is few, if any, in this case. In the irritant contact dermatitis induced by 5% DNCB, Silberberg, Baer, and Rosenthal have reported by electron microscopy that the epidermal cell damages are observed also at 6 hr [2], though not accompanied with apposition of mononuclear cells to Langerhans cells. In our previous study of the irritant contact dermatitis induced by 4% DNCB [10], we demonstrated the appearance of C3-dependent neutrophil chemotactic factors in the lesion after 6 hr. L-fucose exibited no suppressive effect on the irritant contact dermatitis induced by 4% DNCB in the present study. Then, the inflammatory processes of allergic contact dermatitis seem to be independent from those of irritant contact dermatitis.

The present experiments demonstrated that L-fucose, which is a monosaccharide, could inhibit allergic contact dermatitis.

L-fucose did not show significant toxicity to animals so far we examined. In addition, it has been reported that normal human serum contains L-fucose in a small amount [15]. Thus, L-fucose might be able to use for the regulation of human allergic contact dermatitis. Moreover, L-fucose showed no suppressive effect on irritant contact dermatitis and one might be able to differentiate allergic contact dermatitis from irritant contact dermatitis from the effectiveness of L-fucose on the lesion.

REFERENCES

1. Miescher G: Abgrenzung des allergischen und toxischen Geschehens in morphologischer und funktioneller Sicht. Arch Klin Exp Dermatol 213:297–313, 1961
2. Silberberg I, Baer RL, Rosenthal SA: The role of Langerhans cells in contact allergy. I. An ultrastructural study in actively induced contact dermatitis in guinea pigs. Acta Dermatovener (Stockh) 54:321–331, 1974
3. Hunziker N, Winkelmann RK: Langerhans cells in contact dermatitis of the guinea pig. Arch Dermatol 114:1309–1313, 1978
4. Bandmann HJ: Beitrag zur histopathologie allergischer epikutaner Testreaktionen. Hautarzt 11:258, 310, 355, and 393, 1960
5. Remold HG: Requirement for α-L-fucose on the macrophage membrane receptor for MIF. J Exp Med 138:1065–1076, 1973
6. Rocklin RE: Role of monosaccharides in the interaction of two lymphocyte mediators with their target cells. J Immunol 116: 816–820, 1976
7. Amsden A, Ewan V. Yoshida T, Cohen S: Studies on cellular receptors for lymphokines. I. Interaction of chemotactic factors with monosaccharides. J Immunol 120:542–549, 1978
8. Baba T, Yoshida T, Yoshida T, Cohen S: Suppression of cell-mediated immune reactions by monosaccharides. J Immunol 122: 838–841, 1979
9. Chase MW: Experimental sensitization with particular reference to picryl chloride. Int Arch Allergy 5:163–191, 1954
10. Baba T, Tazaki K, Sonozaki H, Torisu M: A neutrophil chemotactic factor and its inhibitor found in DNCB-induced skin inflammatory lesions. J Immunol 118:762–768, 1977
11. Ward PA, Hill JH: Biologic role of complement products. Complement-derived leukotactic activity extractable from lesions of immunologic vasculitis. J Immunol 104:535–543, 1970
12. Stingl G, Katz SI, Clement L, Green I, Shevach EM: Immunologic functions of Ia-bearing epidermal Langerhans cells. J Immunol 121:2005–2013, 1978
13. Stingl G, Wolff-Schreiner EC, Pichler WJ, Gschnait F, Knapp W, Wolff K: Epidermal Langerhans cells bear Fc and C3 receptors. Nature 268:245–246, 1977
14. Stingl G, Katz SI, Shevach EM, Wolff-Schreiner EC, Green I: Detection of Ia antigens on Langerhans cells in guinea pig skin. J Immunol 120:570–578, 1978
15. Tatsumura T, Sato H, Mori A, Komori Y, Yamamoto K, Fukatani G, Kuno S: Clinical significance of fucose level in glycoprotein fraction of serum in patients with malignant tumors. Cancer Res 37:4101–4103, 1977

ACKNOWLEDGEMENT

In order for The Society for Investigative Dermatology to generate additional funds and further expand its activities in the field of Dermatology, a new class of membership, Corporate Sustaining Membership, has been established. The Society wishes to acknowledge the support of the following companies, who are Corporate Sustaining Members:

BURROUGHS WELLCOME COMPANY
DERMIK LABORATORIES, INC.
HOFFMAN-LAROCHE, INC.
ELI LILLY AND COMPANY
HOECHST-ROUSSEL PHARMACEUTICALS, INC.
NEUTROGENA CORPORATION
OWEN LABORATORIES
ORTHO PHARMACEUTICALS, INC. (DERMATOLOGICS DIVISION)
PROCTER AND GAMBLE COMPANY
REED AND CARNRICK PHARMACEUTICALS
RICHARDSON MERRELL, INC. (VICK TOILETRIES DIVISION)
SCHERING LABORATORIES
STIEFEL LABORATORIES, INC.
SQUIBB INSTITUTE FOR MEDICAL RESEARCH
SYNTEX LABORATORIES
UPJOHN COMPANY

The Society also wishes to acknowledge Westwood Pharmaceuticals for its support of Resident/Fellow Memberships in the United States and Canada.